

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
25 April 2002 (25.04.2002)

PCT

(10) International Publication Number
WO 02/32591 A2

- (51) International Patent Classification⁷: **B05D 7/24**
- (21) International Application Number: PCT/DK01/00689
- (22) International Filing Date: 18 October 2001 (18.10.2001)
- (25) Filing Language: English
- (26) Publication Language: English
- (30) Priority Data:
PA 2000 01564 19 October 2000 (19.10.2000) DK
- (71) Applicant (*for all designated States except US*): **PI-COSEP A/S** [DK/DK]; Forskerparken 10, DK-5230 Odense M (DK).
- (72) Inventor; and
- (75) Inventor/Applicant (*for US only*): **WINTER-JENSEN, Bjørn** [DK/DK]; Hornemannsgade 17, DK-2100 Copenhagen Ø (DK).
- (74) Agent: **HEGNER, Annette**; NKT Research & Innovation A/S, Group IP, Danmarks Tekniske Universitet, Diplomvej, Bldg 373, DK-2800 Lyngby (DK).
- (81) Designated States (*national*): AE, AG, AL, AM, AT, AT (utility model), AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, CZ (utility model), DE, DE (utility model), DK, DK (utility model), DM, DZ, EC, EE, EE (utility model), ES, FI, FI (utility model), GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SK (utility model), SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.
- (84) Designated States (*regional*): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).
- Published:
— *without international search report and to be republished upon receipt of that report*
- For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.*

(54) Title: A MATERIAL USEFUL FOR THE SEPARATION OF ORGANIC COMPOUNDS, PROCESSES FOR ITS PREPARATION AND USE OF THE MATERIAL

(57) Abstract: The invention relates to a material comprising a sheet-like substrate and a polymer gradient coating on at least a part of the surface of the substrate, wherein the coating representing a surface composition gradient, such as a pH gradient or a hydrophilicity/hydrophobicity gradient in at least one direction along the surface of the substrate. The invention also relates to a process for the production of the material with the substrate comprising the steps of i. providing the sheet-like substrate; ii. providing monomers for the polymer gradient layer; iii. applying the monomers onto the surface of the substrate; and iv. bringing the monomers to polymerises, where the monomers being applied onto the surface so that the layer or the composition of monomers varies continuously or stepwise in at least one direction along the surface of the substrate to thereby provide a surface composition gradient. The method may preferably include applying the polymer gradient coating using a plasma process.

WO 02/32591 A2

A MATERIAL USEFUL FOR THE SEPARATION OF ORGANIC COMPOUNDS, PROCESSES FOR ITS PREPARATION AND USE OF THE MATERIAL

FIELD OF THE INVENTION

The present invention relates to a material, e.g. a material useful for the separation of
5 organic compounds, such as proteins and biomolecules. The invention also relates to a
method of producing said material as well as a use of said material for the separation of
organic compounds, in particular proteins.

BACKGROUND OF THE INVENTION

Separation of proteins from a complex mixture has traditionally been performed by
10 utilising chromatographic techniques or gel electrophoresis techniques. Gel
electrophoresis techniques are however time and labour consuming and often involve
problems with respect to resolution.

The objective of the present invention is to provide a new method as well as the material
for performing the method, and a method of producing said material, by use of which
15 method it is possible to perform separation of one or more organic compounds from a
mixture of compounds with a high resolution, and which method further is easy and
relatively fast to perform.

BRIEF DESCRIPTION OF THE INVENTION

This and other objectives have been achieved by the invention as it is defined in the
20 claims.

The invention is based on the idea of separating organic compounds on a substrate
comprising a chemical variation of compounds resulting in a composition gradient. The
term surface composition gradient thus means that the surface has a variation in chemical
composition varying along the surface in a graduating pattern, preferably so that the
25 composition in the form of a mixture of two or more compounds is varied with respect to
the amount of the respective compounds stepwise or continuously along the surface of
the substrate, preferably in one or more defined directions. The surface preferably has an
attractive force variation along the surface in a stepwise or continuously graduating
pattern, where an attractive force variation means a variation in the surface's attractive

force property relative to organic compounds, such as a variation in pH value or a variation in hydrophobicity/hydrophilicity.

DESCRIPTION OF THE FIGURE

5 The figure illustrates a plasma reaction chamber 1, which is useful in the process according to the invention. The reaction chamber 1, also designated a vacuum chamber, comprise a winding system 2 in the form of two reels onto which a sheet-like substrate in the form of a tape or similar can be wound during the application of a coating. The system comprises a number of supply units 4 comprising the monomers e.g. as indicated
10 in the form of acid, basic, and neutral components. Each supply unit comprises a flow control 3 for controlling the respective amount of monomer added to the reaction chamber. The reaction chamber 1 comprises two electrodes, 6, 7 which may be of stainless steel. In the shown reaction chamber one of the electrodes 6 is constituted by the outer wall and the other electrode is constituted by grid placed in a distance from the
15 wall. An isolating material 5 e.g. of PE is placed around the electrode wall 6. A substrate 8 is wound from one of the reels to the other during the coating. The plasma 9 is generated in the reaction chamber, and monomer as well as inert gasses such as Ar is added as indicated by the arrows 10, 11.

DETAILED DESCRIPTION OF THE INVENTION

20 The material according to the invention comprises a sheet-like substrate. The term sheet-like substrate means any substrate having a 3-dimensional shape, wherein the substrate in at least one of its dimensions, designated the length, and measured at its longest point is more than, preferably more than 10 times, more preferably more than 100 times its shortest dimension, designated its thickness, and measured in its shortest point.
25 Preferably the substrate in its dimension designated its thickness, and measured in its shortest point, is less than 0.5 times its other 2 dimensions measured in their longest points, preferably less than 0.1 times its other 2 dimensions. The sheet-like substrate preferably includes tapes, bands, strips, felts, sheets, non-woven structures, woven structures, membranes, films, plates, etc. having regular or irregular dimensions. In one
30 particularly interesting embodiment, the sheet-like substrate is a tape roll, which can have

a length of up to several meters. The sheet-like substrate may also include a hollow pipe with an inner surface and an outer surface.

In the embodiment where the sheet-like substrate is in the form of a hollow pipe with an inner surface and an outer surface, the hollow pipe corresponds to a folded sheet, and the
5 hollow pipe may preferably have the dimensions mentioned in the following converted to such folded sheet.

Generally and typically, however, the ratio between the "longest" dimension and the "shortest dimension" (of the three dimensions) is at least in the order of 10, in particular at least in the order 100. The "shortest" dimension is typically referred to as the "thickness"
10 and the "longest" dimension (usually perpendicular to the thickness) is typically referred to as the "length". For sheet-like substrates having regular dimensions, the third dimension is often referred to as the "width". The length and the width are normally of about the same order of magnitude. As an example, the thickness may be in the range of 50-500 μm whereas the width may be in the range of 3-300 mm.

15 The substrate may in one embodiment be in the form of a cord.

In one preferred embodiment the sheet like substrate is in the form of a three dimensional unit, wherein one dimension, designated the length is more than 2 times, preferably more than 5 times and even more preferably more than 10 times the longest of the other two dimension, preferably the length being at least 10 cm such as 25 or 50 cm more
20 preferably at least 100 cm and even more preferably at least 200 cm.

Generally it is preferred that the shortest dimension, designating thickness is between 1 μm and 1 mm, more preferably between 50 and 500 μm . The dimension designating the width, may preferably be between 1 and 1000 mm, more preferably between 3 and 300 mm.

25 The sheet-like substrate comprise a polymer gradient coating on at least a part of the surface of the substrate, said coating representing a surface composition gradient in at least one direction along the surface of the substrate or the material.

The surface composition gradient thus means that the surface has a variation in chemical composition varying along the surface in a graduating pattern, preferably so that the
30 composition is varied in concentration of one or more components and/or in the form of a

mixture of two or more compounds which is varied with respect to the amount of the respective compounds stepwise or continuously along the surface of the substrate.

- The sheet like substrate should preferably comprise a surface area, such as an area of between 0.1 and 100 cm², more preferably between 1 and 20 cm², wherein said part of
- 5 the substrate surface, which preferably may be substantially plane, is coated with the polymer gradient coating is or includes said substantially surface area. The polymer gradient coating should preferably constitute or represent a surface composition gradient essentially parallel to the plane of the coated surface of the substrate. The polymer gradient coating may preferably constitute a surface composition gradient in one direction
- 10 essentially parallel to the plane of the surface of the substrate. Alternatively the polymer gradient coating may constitute a surface composition gradient in two or more directions essentially parallel to the plane of the surface of the substrate, e.g. the surface composition may constitute a surface composition gradient radially extending from a central area or a surface composition gradient extending in two opposite directions.
- 15 Generally it is preferred that the polymer gradient coating constitute a surface composition gradient in one single direction essentially parallel to the plane of the surface of the substrate.

- In one embodiment the substrate comprises a polymer gradient coating which constitute two or more surface composition gradients, such as gradients following each other along
- 20 the same single direction or different gradients in different directions. The two or more surface composition gradients may e.g. be pH gradients with different pH separation values.

- The material according to the invention may e.g. comprise a substrate having one or more of the following gradients: One or more pH value gradients including gradients extending
- 25 at least 0.5 pH units, preferably at least 2 pH units over the substrate surface and/or one or more hydrophilicity/hydrophobicity gradient measured as surface tension, wherein the gradient extending at least 1 dyne/cm, preferably at least 10 dynes/cm and more preferably at least 25 dynes/cm over the substrate surface, more preferably the gradient extension on the substrate is within 10-80 dynes/cm.

- 30 Generally it is preferred that at least 20 %, preferably at least 40 % of the surface of the sheet like substrate being covered with the polymer gradient coating. In many instances,

such as those illustrated in the examples, the coating is present on essentially the total surface of the substrate.

In a preferred embodiment of the invention where the sheet like substrate is in the form of a tape or strip having a thickness with a thickness surface, a first and a second side with, respectively, a first and a second surface, at least one of said thickness surface and first and second surfaces is partly or totally covered with the polymer gradient coating. In this embodiment it is preferred that at least one of said first and second surfaces is partly or totally covered with the polymer gradient coating, even more it is preferred that essentially the whole of at least one of said first and second surfaces is covered with the polymer gradient coating. In this embodiment it is most preferred that at least an elongated surface area of the tape or strip is coated, and that the coating constitute or represent a surface composition gradient in one direction essentially parallel to the plane of the coated surface, where the direction preferably being essentially parallel to the longest line between boarder lines of the elongated surface area. The elongated surface area may preferably be one of the major surfaces of the tape or strip.

In one embodiment where the sheet-like substrate is in the form of a cord, said cord preferably having a round or angular cross-section, such a triangular or rectangular, the cord comprise a coating on its surface extending along the whole or part of the length of the cord and the coating preferably constitute a surface composition gradient in one direction essentially following the length of the cord. Preferably the cord has a substantially circular cross-section, with a diameter of 1-10 mm, preferably between 2 and 4 mm.

In the embodiment where the sheet-like substrate is in the form of a hollow pipe, it is preferred that the inner surface of the hollow pipe is coated with a polymer gradient coating.

Generally it is preferred that the surface composition gradient is in one direction which preferably is a direction following the length direction of the sheet-like substrate, such as a direction substantially parallel to the longitudinal axis of the sheet-like substrate.

The material according to the invention may be constituted of the sheet-like substrate inclusive the polymer gradient coating and any pre- or top-coating.

Alternatively the material according to the invention may, besides the sheet-like substrate inclusive the polymer gradient coating and any pre- or top-coating, comprise a supporting element for the substrate. The supporting element may in principle be any type of supporting element, which does not destroy or totally mask the polymer gradient coating.

- 5 It is preferred that the supporting element is shaped so that the total material preferably is essentially sheet-like, wherein the term sheet-like material means a material having a shape as a sheet-like substrate as defined above.

Generally it is preferred that the supporting element is in the form of a supporting sheet selected from the group consisting of polymers, such as polyolefins including polyethylene
10 (PE) and polypropylene (PP); polytetrafluoroethylene (PTFE); tetra-fluoroethylene-hexafluoropropylene-copolymers (FEP); polyvinyl-difluoride (PVDF); polyamides; polyvinylchloride (PVC); rubbers such as silicon rubbers; glass; paper; carbon fibres; ceramics; metals or mixtures or combinations thereof.

The sheet-like substrate may have any chemical composition such as polymers, e.g.
15 polyolefins, such as polyethylene (PE) and polypropylene (PP), or other thermoplastics, such as polytetrafluoroethylene (PTFE), tetra-fluoroethylene-hexafluoropropylene-copolymers (FEP), polyvinyl-difluoride (PVDF), polyamides (e.g. nylon-6.6 and nylon-11), and polyvinylchloride (PVC), rubbers e.g. silicon rubbers, glass, paper, carbon fibres, ceramics, metals, mixtures thereof etc. The material may further be made from or include
20 any type of fibers and fiber enforced materials. The sheet like substrate comprising the polymer gradient coating may preferably be porous, optionally with a porosity of at least 5 %, preferably at least 10 % by volume. The density of the material should preferably be below 0.9 g/cm³, more preferably below 0.5 g/cm³, in dry and non-compressed state. The substrate may e.g. be made from or include fibers or foam. Preferably the substrate
25 comprise pores or openings, which provides the substrate with a capillary effect to water.

Such materials may be in any suitable form, preferably representing a high surface area. The currently most interesting examples are polyethylene or polypropylene felts, paper, non-woven glass fibres, etc. It is envisaged that particularly interesting sheet-like substrates are polyethylene (EP), polypropylene (PP) or PE/PP felts. The material may
30 include any type of fibers and fiber enforced materials. As will become apparent in view of the following, the sheet-like substrate will in the preferred embodiment constitute all or the main part of the material or sheet-like material. Thus, the mechanical properties (e.g.

flexibility, strength, etc.) of the material will to a great extent be determined by the selection of the sheet-like substrate.

The substrate may be pre-coated in order to modify the properties thereof, e.g. the ability of the surface to adhere to polymer gradient layer or the hydrophobic properties of the substrate as such. The pre-coating may preferably be in the form of a substantially uniform layer or it may alternatively be applied in a pattern such as a pattern providing a gradient as described in the following. Preferably, the pre-coating is in the form of a substantially homogenous layer of a polymer onto the native surface of the substrate. The pre-coating may e.g. be performed in order to moderate the effect of the composition gradient. Thereby it may be possible to obtain an even higher resolution when separating organic compounds.

The polymer gradient coating may in principle have any thickness, but as it will be clear to the skilled person a too thin layer or a too thick layer may give a poor resolution and/or a prolonged separation time. Preferably the thickness of the polymer gradient layer is at least 5 nm, preferably in the range of up to about 100 μm , more preferably in the range of 10-1000 nm. In the most preferred embodiment the polymer gradient coating has a thickness of at the most 5000 nm. The thickness of the layer as such may of course vary over the coated part of the surface of the substrate in accordance with the requirement for a composition gradient. Alternatively, and equally applicable, is a substantially uniform layer thickness, such as a thickness of the polymer gradient coating which is essentially the same though out the coating area.

In one embodiment of the invention the polymer gradient coating varies in thickness continuously or step wise along its direction or directions of surface composition gradient. The polymer gradient coating preferably varies in thickness essentially continuously along its direction or directions of surface composition gradient.

As it has been described above the polymer coating gradient may be in the form of a polymer coating which varies in chemical composition so that it varies along the surface of the substrate in a graduating pattern, preferably so that the composition is varied in concentration of one or more components and/or in the form of a mixture of two or more compounds which is varied with respect to the amount of the respective compounds stepwise or continuously along the surface of the substrate.

- The coating discussed above represents the composition gradient, which is a characteristic feature of the present invention. In essence, the coating provides a layer in the form of a polymer or polymeric composition as further described below, which does not have the same composition or concentration over the complete coated surface. The
- 5 term "composition gradient" or "surface composition gradient" is intended to mean a variation of chemical composition over a surface in a more or less gliding manner. The variation can be expressed as the variation in density of one or more chemical groups. The variance (gradient) may be steep or flat, or may even be step-wise so as to express a virtual gradient.
- 10 The polymer gradient coating comprises one or more chemical components also designated monomers. The term monomers mean components for the polymeric composition to provide the polymer gradient coating. The term monomers should in the present invention include any polymerisable component but it may also include components, which is not polymerisable. Thus the coating may include monomers, which
- 15 is not polymerised but is bound e.g. by an adhesive component. The polymer gradient coating should preferably comprise or be made from at least 10 % by weight, more preferably at least 25 % by weight of polymerisable monomers. In one embodiment the polymer gradient coating consist essentially of polymerised monomers or is made from polymerisable monomers. Monomers which is not polymerised or polymerisable should
- 20 preferably be fixed in or to the coating to avoid unintended migration of these components. As it will be clear to the skilled person the components in the polymer gradient coating may be any type of component, wherein preferably at least one of the components in the coating should have an effect of the migration of at least one of the organic compounds in a mixture that is to be separated, such as proteins.
- 25 The variance in composition may for example represent a pH gradient, a hydrophobicity gradient, a polarity gradient, a surface tension gradient, a biocompatibility gradient, a binding affinity gradient, etc. A pH gradient is particularly relevant where the material is utilised in a method for the separation of proteins.

- The composition gradient is generally in a direction substantially parallel to the plane of
- 30 the material or sheet-like substrate. The "plane of the material or sheet-like substrate" refers to the macroscopic plane of the sheet, film, tape, etc. Thus, normally (in particular for films, strips and tapes) the composition gradient may as mentioned preferably be in a direction substantially parallel to the longitudinal axis of the material.

In an embodiment wherein the polymer gradient coating varies in composition continuously or step wise along its direction or directions of surface composition gradient, and the polymer gradient coating varies in composition essentially continuously along its direction or directions of surface composition gradient, it is preferred that the is composed
5 from two or more monomers with varying amounts of the respective monomers in the polymer gradient composition along the direction or directions of surface composition gradient.

In one preferred embodiment the polymer gradient coating comprise acidic groups. In this embodiment it is particularly preferred that the polymer gradient coating being made from
10 monomers including monomers selected from the group consisting of acrylic acid, methacrylic acid and vinylacetic acid.

In one preferred embodiment the polymer gradient coating comprise alkaline groups. In this embodiment it is particularly preferred that the polymer gradient coating being made from monomers including monomers selected from the group consisting of cyanoacrylate,
15 cyanomethacrylate, ethylene diamine and allylamine.

In one preferred embodiment the polymer gradient coating comprise neutral groups, such as pH neutral groups. In this embodiment it is particularly preferred that the polymer gradient coating being made from monomers including monomers selected from the group consisting of propylene, ethylene, styrene, methyl vinyl ether, hexene,
20 ethyleneglycolvinylether, diethyleneglycolvinylether and vinylpyrrolidone.

In a particularly preferred embodiment the composition gradient is a pH gradient, and it is preferred that the polymer gradient coating is made from monomers including at least mixtures of acrylic acid and cyanoacrylate, mixtures of acrylic acid and ethylene diamine, mixtures of acrylic acid and allylamine, or mixtures of vinylacetic acid and allylamine.

25 In another particularly preferred embodiment the composition gradient is a hydrophobicity gradient, and in this embodiment it is preferred that the polymer gradient coating is made from monomers comprise a mixture of a monomer being relatively hydrophobic preferably selected from the group consisting of perfluorohexene, perfluoromethylpentene, hexene, pentene, cyclohexene, acetylene, styrene, vinylbornene, and mixtures thereof, and a
30 monomer being relatively hydrophilic preferably selected from the group consisting of vinylacetate, vinylpyrrolidone, ethyleneglycolvinylether, diethyleneglycolvinylether, methacrylate, methylmethacrylate, allyl alcohol and mixtures thereof.

The substrate may further comprise a top-coating, which e.g. may be a polymeric coating, such as a top-coating in the form of a polymer layer with a thickness of below 20 nm, preferably below 5 nm, and more preferably between 0,5 and 3 nm. This coating may be provided to protect the polymer gradient coating e.g. from CO₂, O₂ or any other gases that
5 unintentionally may react or block the components in the polymer gradient coating, or the top-coating may modified or moderated the composition gradient of the material. In this way, the functional groups of e.g. a pH gradient (i.e. acidic and basic groups, may not bind so strongly to the molecules intended for separation.

The material according to the invention is particularly useful for the separation of a mixture
10 of organic compounds, in particular proteins.

The process

The present invention also provides a novel process for the manufacture of the materials having on the surface thereof a composition gradient as defined in the claims. The process according to the invention comprise the steps of:

15

- i. providing the sheet-like substrate;
- ii. providing monomers for the polymer gradient layer;
- iii. applying the monomers onto the surface of the substrate; and
- iv. bringing the monomers to polymerises;

20

the monomers being applied onto the surface so that the layer or the composition of monomers varies continuously or stepwise in at least one direction along the surface of the substrate to thereby provide a surface composition gradient.

The step iv of bringing the monomers to polymerises, means that a least some of the
25 monomers is broad to reaction or polymerisation, e.g. such at as least 10 %, preferably at least 20 % and more preferably at least 40 % by weight of the monomers. Basically it is preferred that the polymerisation is sufficient to provide a solid layer which is not soluble in an aqueous solvent having a pH value between 4 and 10.

The monomers may be applied and polymerised using any method. The skilled person
30 will be able so find a method which is adequate for the given monomer composition.

It should be understood that the selection of monomers the thickness of coating layer(s), the selection of substrate and material and e.t.c. are as described above for the material according to the invention.

In principle the monomers may be applied onto the surface and broad to polymerises
5 using any conventional methods e.g. by using a coating process including applying the monomer with a catalyst and initiating a free radical polymertsation. Another preferred method is the plasma process which will be described in detail below:

Coating process

Examples of such general deposition/polymerisation processes are described in the
10 recent patent applications by Creavis, see e.g. EP 1 018 531 A2, WO 00/44818, EP 1 040 874 A2, which are hereby incorporated by reference. The deposition of monomers should of course be controlled by suitable means in order to provide the composition gradient.

The coating process includes vaporisation coating wherein coating process, the steps iii and iv of applying the monomers onto the surface of the substrate; and bringing the
15 monomers to polymerises; being carried out by evaporation of the monomers, deposition of the monomers and optionally a catalyst onto the substrate to provide a gradient, and bringing the monomers to polymerises by free radical polymerisation of the monomers to form the polymer coating in such a manner that the composition gradient is prepared.

The monomer should basically be vaporisable for this process. The catalyst may in
20 principle be any type of catalyst useful for providing a sufficiently polymerisation. The catalyst may be applied in a separate step or simultaneously with the other components. The composition gradient may be provided by use of masking as described above under the plasma process.

Plasma process

25 The plasma process may preferably comprise the steps of:

- 1) providing the sheet-like substrate;
- 2) providing a plasma reaction chamber;
- 3) moving the substrate through the reaction chamber in a direction substantially parallel
30 to the plane of the substrate; and
- 4) feeding a coating gas into the reaction chamber;

the plasma reaction chamber, the movement of the substrate and the feeding of the coating gas providing conditions so that the coating gas is allowed to deposit and polymerise on the surface of the substrate; wherein the deposition and polymerisation
5 conditions are varied in response to the position of the sheet-like substrate in the plasma reaction chamber so as to provide the composition gradient.

According to a preferred process of the invention it is preferred that the steps iii and iv of applying the monomers onto the surface of the substrate; and bringing the monomers to polymerises as defined in the claims; being carried out using a plasma polymerisation
10 process including

- a. providing a plasma reaction chamber;
- b. exposing the surface to be coated to the reaction zone in the reaction chamber;
- c. feeding a coating gas into the reaction chamber;

15

providing conditions so that the coating gas is allowed to deposit and polymerise on the surface of the substrate.

Plasma chemical vapour deposition (CVD) methods for modification of surfaces are known in the art. US 5,643,638 describes a method for producing a gradient layer in the
20 direction of the layer growth. The technique was developed for modification of the mechanical properties of optical lenses. US 5,217,749 describes a method of making polymer thin films for optical uses by utilising plasma polymerisation techniques. The method is particularly relevant for depositing layers having different refractive indices on optical lenses, optical wave guides or in optical integrated circuitry.

25 PH gradients in gels have been provided for polyacrylamide matrices as described in WO 93/11174 and WO 97/16462.

The skilled person may find further information concerning plasma polymerisation, and the document is hereby incorporated by reference.

The sheet-like substrate can be of any format and size as described above, and the
30 substrate may comprise a pre-coating e.g. in the form of a plasma-coating.

The layer of plasma polymerised coating is deposited on the sheet-like substrate in a manner so as to provide the composition gradient in thicknesses as described above.

The coating layer should as mentioned be present on at least a part of the surface of the substrate, typically on at least 20% of the surface area, such as at least 40% of the surface area. In many instances, such as those illustrated in the examples, the coating is present on essentially the total surface of the substrate. In the instances where only a fraction of the surface of the substrate should be covered, the plasma reaction chamber may include means for covering/masking the part of the surface where the coating is undesirable/unnecessary, or the substrate may be covered by a material (e.g. an adhesive tape) which subsequently can be detached from the substrate. Generally, both sides of a substrate is coated, but it is possible to avoid coating of the one side of the substrate by the means indicated above or by means easily derived by the person skilled in the art.

As will be understood from the description below and the application in general, the direction of the gradient may preferably be determined by a movement of the substrate through the plasma reaction chamber during the application of polymer gradient coating.

The plasma reaction chamber can basically be of any conventional type, however it should be possible to adapt means for moving the substrate through the reaction chamber. One example of a suitable reaction chamber is outlined in the figure. An other possible reaction chamber is the one described by the applicant in the earlier WO 00/44207 or those utilising the electrode system described in EP 0 741 404 B1. These publications are hereby incorporated by reference.

The plasma generated should have a level of intensity which allows a substantial portion of the functional groups, e.g. acidic groups, basic groups, lipophilic group, etc. to be preserved. It is possible to utilise a 2 or 3 phase AC plasma which offers the possibility of using a sufficiently low energy, e.g. energy levels of less than 10 W/l such as less than 5 W/l or even less than 2 W/l. Alternatively, a pulsed RF plasma can be used, e.g. of 20-100 ms on-time per second.

The pressure in the reaction chamber should preferably be in the range of 10-1000 μ bar, such as 50-200 μ bar. The pressure in the reaction chamber is controlled by a vacuum pump, and a supply of an inert gas and the coating gas. The inert gas is suitably a noble gas such as helium, argon, neon, krypton or a mixture thereof. As an example, the

combination of energy level and pressure could be so that the pressure is about 50-200 μ bar and the energy level should be less than 6 W/l for a situation where the coating gas comprises sensitive monomers.

Hence, the step of providing a plasma reaction chamber will be performed in accordance
5 with the instructions given herein with possible modification obvious for the person skilled in the art.

In one embodiment the step of moving the part of the substrate on which the plasma polymerisation is intended relative to the reaction chamber is essential for the process according to the invention. The substrate is typically moved in a direction substantially
10 parallel to the plane of the substrate. More precisely, the substrate is moved in the direction corresponding to the direction of the desired gradient. It should be understood that the substrate may be moved in an angled direction with respect to the plane of the substrate (e.g. up to 30° off the parallel to the plane), however this will normally not provide any significant benefits.

15 In an alternative approach, the sheet-like substrate may be more or less stationary relative to the reaction chamber. In this alternative embodiment, the sheet-like substrate may be masked so that only an only a part of the substrate is exposed to the coating gas (e.g. through a slot in the "mask"). The "mask" may then be moved relative to the reaction chamber in order to provide the composition gradient. In this embodiment it is preferred
20 that the surface to be coated is being exposed to be coated in the reaction zone by moving the substrate through the reaction chamber or placing the substrate in the reaction chamber with its surface partly or totally masked, and moving the mask continuously or stepwise to expose the surface to be coated. The movement of the mask may preferably be in a direction substantially parallel to the plane of the substrate; and the movement of
25 the mask and the feeding of the coating gas providing conditions so that the coating gas is allowed to deposit and polymerise on the surface of the substrate; wherein the deposition and polymerisation conditions are varied in response to the position of the mask on the sheet-like substrate in the plasma reaction chamber so as to provide the composition gradient.

30 The feeding of the substrate may preferably be conducted in a continuous manner so as to provide a uniform gradient, however it is possible to vary the speed (see below) or to move the sheet-like substrate in a step-wise manner.

The sheet-like substrate can be moved through the reaction chamber using a conveyor belt to which the sheet-like substrate is clamped, or the sheet-like substrate may be attached to a tape. In an interesting example, which is illustrated in the example section, the sheet-like substrate is provided in the form of a tape, which is spooled between two
5 reels. In this way a relatively long and broad band is provided which can be cut into finer tapes or sheets. Thus, generally, the prepared material may subsequently be divided into two or more smaller pieces.

In the further important step, a coating gas is fed into the reaction chamber. The coating gas may comprise one or more monomers participating in the plasma polymerisation
10 process; preferably two or more monomer types are used. It is envisaged that the feeding can be conducted by using "conventional" means such as flow controllers, gas inlets, etc., however as will be apparent from following, the accurate control of the feeding process is often quite important for the overall process. The feeding may be continuous or varied, or step-wise, e.g. in phase with the step-wise feed of the substrate, etc. The location of feed
15 nozzles, etc. will determine where and how efficient the coating gas is deposited as will be appreciated by the person skilled in the art.

In order to achieve the composition gradient on the surface of the material, the plasma reaction chamber, the movement of the substrate, and the feeding of the coating gas may preferably in combination provide conditions so that the coating gas is allowed to deposit
20 and polymerise on the surface of the substrate; wherein the deposition and polymerisation conditions are varied in response to the position of the sheet-like substrate in the plasma reaction chamber so as to provide the composition gradient.

One quite important element in the plasma polymerisation process is the coating gas. As mentioned above, the coating gas may comprise one or more monomers. In some
25 interesting embodiments, the coating gas comprises at least two monomers and preferably at least one functional group monomer. The functionalities of the monomers may deliberately be chosen so that the functionality is transformed in the plasma polymerisation process, e.g. as described and illustrated below for the cyanoacrylate which transforms to acrylamide in the course of the plasma polymerisation process. Due
30 to the fact that a substantial amount of the monomers should survive (or at least not lose the desired functional group) in the plasma until polymerisation on the substrate, it is preferred that monomers having relatively low molecular weights are utilised, e.g. a molecular weight of less than 600, preferably less than 450.

In the instances where a pH gradient is desirable, it is relevant to utilise at least one type of monomer providing an acidic group (e.g. acrylic acid, methacrylic acid, vinylacetic acid, etc.) and/or at least one type of monomer providing a basic group (e.g. cyanoacrylate, cyanomethacrylate, ethylene diamine, allylamine, etc.). Furthermore, it is sometimes
5 relevant also to include a monomer which only provides a substantially neutral group so as to moderate the density of the acidic and/or basic groups on the coating. (The term "providing" is used in order to express that the functional group may be "masked" in the monomer used as starting material (e.g. as for cyanoacrylate).)

As an example, it is envisaged that a coating gas for providing a pH gradient may
10 comprise a mixture of a monomer providing an acidic group and a monomer providing a basic group, e.g. mixtures of acrylic acid and cyanoacrylate, mixtures of acrylic acid and ethylene diamine, mixtures of acrylic acid and allylamine, or mixtures of vinylacetic acid and allylamine.

These mixtures may furthermore include monomers which only provide substantially
15 neutral groups, e.g. propylene, ethylene, styrene, methyl vinyl ether, hexene, ethyleneglycolvinylether, diethyleneglycolvinylether, vinylpyrrolidone, etc.

As another example, it is envisaged that a coating gas for providing a hydrophobicity gradient, a polarity gradient, or a surface tension gradient may comprise a mixture of a monomer being relatively hydrophobic (e.g. perfluorohexene, perfluoromethylpentene,
20 hexene, pentene, cyclohexene, acetylene, styrene, vinylbornene, etc.) and a monomer being relatively hydrophilic (e.g. vinylacetate, vinylpyrrolidone, ethyleneglycolvinylether, diethyleneglycolvinylether, methacrylate, methylmethacrylate, allyl alcohol, etc.).

The deposition and polymerisation conditions are important for the preparation of the composition gradient. By varying one or more parameters in the reaction chamber, e.g.
25 the composition of the coating gas, the partial pressure of the coating gas constituents, the speed at which the substrate is moved through the reaction chamber, the pressure in the plasma reaction chamber, voltage in the plasma reaction chamber, any pulse length, etc., the plasma polymerisation process can be changed to a smaller or larger degree in such a manner that the desired composition gradient is obtained. In one important
30 embodiment, the deposition and polymerisation conditions are varied by varying the composition of the coating gas (see the examples section). In another embodiment, the deposition and polymerisation conditions are varied by varying the speed at which the substrate is moved through the plasma reaction chamber. In a third embodiment, the

deposition and polymerisation conditions are varied by varying the partial pressure of the coating gas. In still another embodiments, the deposition and polymerisation conditions are varied by varying the pressure and/or voltage in the plasma reaction chamber or the pulse width and/or amplitude of the plasma voltage.

- 5 The composition gradient of the material may be further modified or moderated by providing a thin coat of a further polymer on top of the gradient, e.g. by a plasma polymerisation process. In this way, the functional groups of e.g. a pH gradient (i.e. acidic and basic groups, may not bind so strongly to the molecules intended for separation. As it has been shown in the example section, such a layer may be quite thin such as 0.5-20
10 nm.

Industrial application

- The materials of the present invention are according to preliminary experiments particularly useful for the separation of mixtures of organic compounds, e.g. biomolecules
15 such as proteins in application where electrophoresis gels normally are used.

Thus, the present invention also relates to the use of the sheet-formed material as described above for the separation of a mixture of organic compounds, in particular proteins.

EXAMPLES

20 Manufacture of materials

Example 1 - Manufacturing a pH-gradient for separation of proteins

The substrate for the pH-gradient was a polyethylene/polypropylene (PE/PP) felt from Freudenberg (VK1099, 60g/m²), which was available in 30 mm wide rolls.

- The substrate felt was spooled from one reel to another under the plasma polymerisation.
25 Hereby the felt passed through the plasma reaction chamber; about 5 cm of the felt (in the length-wise direction) was exposed in the reaction chamber. The plasma was generated by a 2-phase AC supply.

The plasma polymerisation of the acidic and basic monomers took place in the reaction chamber by simultaneously varying the mixture of acidic and basic monomers. Acrylic acid was used as the monomer having an acidic group and allylamine was used as monomer having a basic group. The acrylic acid was bubbled through with argon.

- 5 The reels for spooling were placed in boxes provided with a flow of argon (slight over pressure in relation to the polymerisation area) to prevent undesirable downstream polymerisation ("protection Ar").

The reaction chamber with reels was essentially constructed as outlined in the figure

Procedure:

- 10 The felt (4 metres) was placed on the winding-equipment (reels). The vacuum chamber was closed and evacuated to a pressure of 10 μ bar. A flow of argon for bubbling the acrylic acid was adjusted to 20 sccm (standard cubic centimetres per minute (ml/min)) with the use of a flow controller. The flow of argon/acrylic acid was adjusted to 40 sccm. The flow of allylamine was adjusted to 3 sccm. The flow of "protection Ar" was adjusted to
15 20 sccm. The pressure was increased to 100 μ bar and was kept constant at this level during the plasma polymerisation. The plasma was turned on with an effect of 2 W/l and was kept constant at this level during the plasma polymerisation.

After 30 sec, the spooling of the felt was started with a speed of 10 cm/min corresponding to a "residence time" in the plasma reaction chamber of 30 sec.

- 20 Simultaneously with the spooling, a computer-controlled change of the acrylic acid and allylamine flows started. The total flow of monomer (combined molar flow of acrylic acid and allylamine) was almost constant. After 30 min of plasma polymerisation (corresponding to 3 metres of felt) the flow of argon/acrylic acid was 25 sccm the flow of allylamine was 15 sccm.
- 25 The plasma was turned off, and after one min the spooling was stopped. All flows, except for that of "protection Ar", were interrupted. After 1 min at this pressure, the flow of "protection Ar" was also interrupted. The pressure was raised to atmospheric pressure and the vacuum chamber was opened. The felt could be removed from the reels.

- The reaction provided a felt having a pH gradient on the surface thereof. By test with a
30 pH-liquid, the strips showed a pH-range from 4.5 to 7.5 which equals to 1 pH-unit per

metre. The thickness of the plasma polymerised layer of acidic and basic monomer was in the range of 30-40 nm. For protein separation, the felt was cut into strips of 3 mm width, spooled and packed.

Example 2 - Modification of pH gradient

- 5 The felt provided with a pH gradient as described in Example 1 was modified in order to make the functional groups of the plasma polymerised coating less susceptible to reaction with the organic molecules to be separated on the surface. This was done by providing a thin layer of a pH inert polymer on top of the polymers representing the pH gradient. This modification procedure followed the above process after the plasma was turned off (i.e. before the "All flows, except for that of "protection Ar", were interrupted") :

All flows, except for "protection Ar", were interrupted. After 2 min, a flow of diethylene-glycolvinylether was applied; flow rate 20 sccm. After another 2 min, the plasma was turned on with an effect of 4 W/l and the spooling was started as "rewinding" with a speed of 50 cm/min. The (re)winding was continued for 6 min. Hereafter the diethyleneglycolvinyl-
15 ether flow was interrupted and the pressure was lowered to 40 μ bar. After 1 min at this pressure, the flow of "protection Ar" was interrupted and the pressure was allowed to raised to atmospheric pressure and the vacuum chamber was opened. The felt could be removed from the reels.

The thickness of the polymerised diethyleneglycolvinylether was around 3-4 nm. By test
20 with a pH-liquid, the strips showed a pH-range from 4.5 to 7.5 which equals to 1 pH-unit per metre.

After the plasma polymerisation the roll could be sliced into strips of 3 mm width for the protein separation.

*Example 3 - Manufacturing a membrane with varying hydrophilicity for the separation of
25 biomolecules*

A two metres long polyethylene (PE) membrane (200 μ m thick, 10 μ m pore size) with a width of 10 cm was placed on reels as described in Example 1. As the hydrophilic monomer was used vinylpyrrolidone bubbled with argon (argon/vinylpyrrolidone) and as the hydrophobic monomer was used hexene.

The membrane was spooled from one reel to another during the plasma polymerisation. Hereby the felt passed through the plasma reaction chamber equipped as described in Example 1.

The plasma polymerisation of the hydrophilic and hydrophobic monomers took place by simultaneously varying the mixture of hydrophilic and hydrophobic monomers.

Procedure:

The vacuum chamber was closed and evacuated to a pressure of 10 μ bar. The flow of argon for bobbling the vinylpyrrolidone was adjusted to 10 sccm by a flow controller. The flow of argon/vinylpyrrolidone was adjusted to 50 sccm. The initial flow of hexene was set to 0 sccm. The flow of "protection Ar" was adjusted to 20 sccm. The pressure was allowed to increase to 100 μ bar and was kept at this level during the polymerisation. The plasma was turned on with an effect of 0.5 W/l and was kept constant at this level during the polymerisation.

After 60 sec the spooling of the felt was started with a speed of 5 cm/min corresponding to a "residence time" in the plasma of 60 sec. Simultaneously with the spooling, a computer-controlled change of the vinylpyrrolidone and hexene flows started. The total flow of monomer (combined molar flow of vinylpyrrolidone and hexene) was almost constant. After 30 min of plasma polymerisation (corresponding to 1.5 metres of membrane), the flows were 11 sccm for argon/vinylpyrrolidone and 40 sccm for hexene.

The plasma was turned off and after one minute the spooling was stopped. All flows, except "protection Ar", were interrupted and the pressure was lowered to 40 μ bar. After 1 min at this pressure, the flow of "protection Ar" was interrupted and the pressure was allowed to raise to atmospheric pressure and the vacuum chamber was opened.

CLAIMS

1. A material comprising a sheet-like substrate and a polymer gradient coating on at least a part of the surface of the substrate, said coating representing a surface composition
5 gradient in at least one direction along the surface of the substrate.
2. A material according to claim 1, wherein the sheet like substrate comprise a substantially plane surface area, wherein said part of the surface of the substrate being coated with the polymer is or includes said substantially plane surface area, said coating representing a surface composition gradient essentially parallel to the plane of the surface
10 of the substrate, preferably said coating representing a surface composition gradient in one direction essentially parallel to the plane of the surface of the substrate.
3. A material according to any one of the claims 1 and 2, wherein the sheet like substrate is in the form of a cord, a hollow pipe or a three dimensional unit, wherein one dimension designated the thickness is shorter than the two other dimensions designated the length
15 and the width, respectively, said sheet like substrate preferably being selected from the group consisting of a tape, a band, a strip, a sheet, a plate and a cord.
4. A material according to any one of the preceding claims, wherein the sheet like substrate is in the form of a three dimensional unit, wherein one dimension designated the length is more than 2 times, preferably more than 5 times and even more preferably more
20 than 10 times the longest of the other two dimension, preferably the length being at least 50 cm more preferably at least 100 cm and even more preferably at least 200 cm.
5. A material according to any one of the preceding claims, wherein the sheet like substrate is in the form of a cord or a three dimensional unit, wherein one dimension designated the thickness is between 1 μm and 1 mm, more preferably between 50 and
25 500 μm .
6. A material according to any one of the preceding claims, wherein the sheet like substrate is in the form of a cord or a three dimensional unit, wherein one dimension designated the width between 1 and 1000 mm, more preferably between 3 and 300 mm.

7. A material according to any one of the preceding claims, wherein at least 20 %, preferably at least 40 % of the surface of the sheet like substrate being covered with the polymer gradient coating.
8. A material according to any one of the preceding claims, wherein the sheet like
5 substrate is in the form of a tape or strip having a thickness with a thickness surface, a first and a second sides with respectively a first and a second surfaces, at least one of said thickness surface and first and second surfaces being partly or totally covered with said polymer gradient coating, preferably at least one of said first and second surfaces being partly or totally covered with said polymer gradient coating, more preferably
10 essentially the whole of at least one of said first and second surfaces being covered.
9. A material according to claim 8, wherein at least an elongated surface area of said tape or strip being coated, and wherein said coating representing a surface composition gradient in one direction essentially parallel to the plane of the coated surface, said direction preferably being essentially parallel to the longest line between boarder lines of
15 the elongated surface area.
10. A material according to according to any one of the preceding claims 1-7, wherein the sheet like substrate is in the form of a cord, said cord preferably having a round or angular cross-section, said cord comprise a coating on its surface extending along the whole or part of the length of the cord said coating representing a surface composition gradient in
20 one direction essentially following the length of the cord.
11. A process according to any one of the preceding claims, wherein the composition gradient is in a direction substantially parallel to the longitudinal axis of the sheet-like substrate.
12. A material according any one of the preceding claims wherein said material essentially
25 is constituted of said substrate.
13. A material according any one of the preceding claims 1-11 wherein said material further comprise a supporting element for the substrate, the total material preferably be essentially sheet-like, more preferably said supporting element being a supporting sheet selected from the group consisting of polymers, such as polyolefins including polyethylene
30 (PE) and polypropylene (PP); polytetrafluoroethylene (PTFE); tetra-fluoroethylene-hexafluoropropylene-copolymers (FEP); polyvinyl-difluoride (PVDF); polyamides;

polyvinylchloride (PVC); rubbers such as silicon rubbers; glass; paper; fibres, such as carbon fibres; fiberreinforced materials; ceramics; metals or mixtures or combinations thereof.

14. A material according any one of the preceding claims wherein said substrate being of
5 a material selected from the group consisting of polymers, such as polyolefins including polyethylene (PE) and polypropylene (PP); polytetrafluoroethylene (PTFE); tetra-fluoroethylene-hexafluoropropylene-copolymers (FEP); polyvinyl-difluoride (PVDF); polyamides; polyvinylchloride (PVC); rubbers such as silicon rubbers; glass; paper; fibres, such as carbon fibres; fiberreinforced materials; ceramics; metals or mixtures or
10 combinations thereof.

15. A material according any one of the preceding claims wherein said polymer gradient coating having a thickness of at the most 5000 nm.

16. A material according any one of the preceding claims wherein said polymer gradient coating having a thickness of at least 5 nm, preferably in the range of up to 100 μm , more
15 preferably in the range of 10-1000 nm.

17. A material according any one of the preceding claims wherein said polymer gradient coating varies in thickness continuously or step wise along its direction or directions of surface composition gradient, said polymer gradient coating preferably varies in thickness essentially continuously along its direction or directions of surface composition gradient.

20 18. A material according any one of the preceding claims wherein said polymer gradient coating varies in composition continuously or step wise along its direction or directions of surface composition gradient, said polymer gradient coating preferably varies in composition essentially continuously along its direction or directions of surface composition gradient, said composition preferably being composed from two or more
25 monomers with varying amounts of the respective monomers in the polymer gradient composition along the direction or directions of surface composition gradient.

19. A material according any one of the preceding claims wherein said coating comprise acidic groups, preferably said polymer gradient coating being made from monomers including monomers selected from the group consisting of acrylic acid, methacrylic acid,
30 and vinylacetic acid.

20. A material according any one of the preceding claims wherein said coating comprise alkaline groups preferably said polymer gradient coating being made from monomers including monomers selected from the group consisting of cyanoacrylate, cyanomethacrylate, ethylene diamine and allylamine.
- 5 21. A material according any one of the preceding claims wherein said coating comprise neutral groups, such as pH neutral groups, preferably said polymer gradient coating being made from monomers including monomers selected from the group consisting of propylene, ethylene, styrene, methyl vinyl ether, hexene, ethyleneglycolvinylether, diethyleneglycolvinylether and vinylpyrrolidone.
- 10 22. A material according any one of the preceding claims, wherein the composition gradient is a pH gradient, preferably said polymer gradient coating being made from monomers including at least mixtures of acrylic acid and cyanoacrylate, mixtures of acrylic acid and ethylene diamine, mixtures of acrylic acid and allylamine, or mixtures of vinylacetic acid and allylamine.
- 15 23. A material according any one of the preceding claims, wherein the composition gradient is a hydrophobicity gradient, preferably said polymer gradient coating being made from monomers comprise a mixture of a monomer being relatively hydrophobic preferably selected from the group consisting of perfluorohexene, perfluoromethylpentene, hexene, pentene, cyclohexene, acetylene, styrene, vinylbornene,
20 and mixtures thereof, and a monomer being relatively hydrophilic preferably selected from the group consisting of vinylacetate, vinylpyrrolidone, ethyleneglycolvinylether, diethyleneglycolvinylether, methacrylate, methylmethacrylate, allyl alcohol and mixtures thereof.
24. A material according any one of the preceding claims, wherein the substrate comprise
25 a pre-coating below all or a part of the polymer gradient coating.
25. A material according any one of the preceding claims, wherein the substrate comprise a top-coating, said top-coating preferably being in the form of a polymer layer with a thickness of below 20 nm, preferably below 5 nm, and more preferably between 0,5 and 3 nm.
- 30 26. A process for the manufacture of a material, according to anyone of the claims 1-25 said process comprising the steps of:

- i. providing the sheet-like substrate;
- ii. providing monomers for the polymer gradient layer;
- iii. applying the monomers onto the surface of the substrate; and
- iv. bringing the monomers to polymerises;

5

the monomers being applied onto the surface so that the layer or the composition of monomers varies continuously or stepwise in at least one direction along the surface of the substrate to thereby provide a surface composition gradient.

27. A process according to claim 26, wherein said steps iii and iv of applying the
10 monomers onto the surface of the substrate; and bringing the monomers to polymerises; being carried out using a plasma polymerisation process including

- a. providing a plasma reaction chamber;
- b. exposing the surface to be coated to the reaction zone in the reaction chamber;
- 15 c. feeding a coating gas into the reaction chamber;

providing conditions so that the coating gas is allowed to deposit and polymerise on the surface of the substrate.

28. A process according to claim 27, wherein said surface to be coated being exposed to
20 be coated in the reaction zone by moving the substrate through the reaction chamber, said movement preferably being in a direction substantially parallel to the plane of the substrate; and the movement of the substrate, and the feeding of the coating gas providing conditions so that the coating gas is allowed to deposit and polymerise on the surface of the substrate; wherein the deposition and polymerisation conditions are varied
25 in response to the position of the sheet-like substrate in the plasma reaction chamber so as to provide the composition gradient.

29. A process according to any one of the claims 27 and 28, wherein said surface to be coated being exposed to be coated in the reaction zone by moving the substrate through the reaction chamber or placing the substrate in the reaction chamber with its surface
30 partly or totally masked, and moving the mask continuously or stepwise to expose the surface to be coated, said movement of the mask preferably being in a direction substantially parallel to the plane of the substrate; and the movement of the mask and the feeding of the coating gas providing conditions so that the coating gas is allowed to

deposit and polymerise on the surface of the substrate; wherein the deposition and polymerisation conditions are varied in response to the position of the mask on the sheet-like substrate in the plasma reaction chamber so as to provide the composition gradient.

30. A process according to any one of the preceding claims 27 -29 wherein the coating
5 gas comprises of two or more types of monomers.

31. A process according to any one of the preceding claims 27 -30, wherein the deposition and polymerisation conditions are varied by varying the composition of the coating gas.

32. A process according to any of the preceding claims 27-30, wherein the deposition and
10 polymerisation conditions are varied by varying the speed at which the substrate is moved through the plasma reaction chamber and/or the deposition and polymerisation conditions are varied by varying the speed at which the mask is moved from the substrate.

33. A process according to any one of the preceding claims 27-32, wherein the deposition and polymerisation conditions are varied by varying the partial pressure of the coating
15 gas.

34. A process according to any one of the preceding claims 27-33, wherein the deposition and polymerisation conditions are varied by varying the pressure and/or voltage in the plasma reaction chamber.

35. A process according to any one of the preceding claims 27-34, wherein the plasma is
20 pulsed, and wherein the deposition and polymerisation conditions are varied by varying the pulse width and/or amplitude of the plasma voltage.

36. A process according to any one of the preceding claims 27-35, wherein the coating gas comprises a monomer which provides an acidic group, said monomers preferably being selected from the group consisting of acrylic acid, methacrylic acid and vinylacetic
25 acid.

37. A process according to any one of the preceding claims 27-36, wherein the coating gas comprises a monomer which provides a basic group, said monomers preferably being selected from the group consisting of cyanoacrylate, cyanomethacrylate, ethylene diamine and allylamine.

38. A process according to any one of the preceding claims 27-37, wherein the coating gas comprises a monomer which provides a substantially neutral group, said monomers preferably being selected from the group consisting of propylene, ethylene, styrene, methyl vinyl ether, hexene, ethyleneglycolvinylether, diethyleneglycolvinylether and
5 vinylpyrrolidone.

39 A process according to any one of the preceding claims 27-38, wherein the composition gradient is a pH gradient, preferably said polymer gradient coating being made from monomers including at least mixtures of acrylic acid and cyanoacrylate, mixtures of acrylic acid and ethylene diamine, mixtures of acrylic acid and allylamine, or
10 mixtures of vinylacetic acid and allylamine.

40. A process according to any one of the preceding claims 27-38, wherein the composition gradient is a hydrophobicity gradient, preferably said polymer gradient coating being made from monomers comprise a mixture of a monomer being relatively hydrophobic preferably selected from the group consisting of perfluorohexene,
15 perfluoromethylpentene, hexene, pentene, cyclohexene, acetylene, styrene, vinylbornene, and mixtures thereof, and a monomer being relatively hydrophilic preferably selected from the group consisting of vinylacetate, vinylpyrrolidone, ethyleneglycolvinylether, diethyleneglycolvinylether, methacrylate, methylmethacrylate, allyl alcohol and mixtures thereof.

20 41. A process according to claim 26, wherein said steps iii and iv of applying the monomers onto the surface of the substrate; and bringing the monomers to polymerises; being carried out by evaporation of the monomers, deposition of the monomers and optionally a catalyst onto the substrate to provide a gradient, and bringing the monomers to polymerises by free radical polymerisation of the monomers to form the polymer
25 coating with a polymer coating gradient.

42. A process according to any of the preceding claims 26-41, wherein the substrate is pre-coated in order to modify the surface thereof, said substrate preferably being pre-coated by plasma polymerisation.

43. A process according to any of the preceding claims 26-42, wherein the sheet-like
30 substrate is post-coated to provide a top-coating, preferably said post-coating results in a modification to thereby make the functional groups of the plasma polymerised gradient coating less susceptible to reaction.

44. The use of the material according to any of the claims 1-25 for the separation of a mixture of organic compounds, in particular proteins.

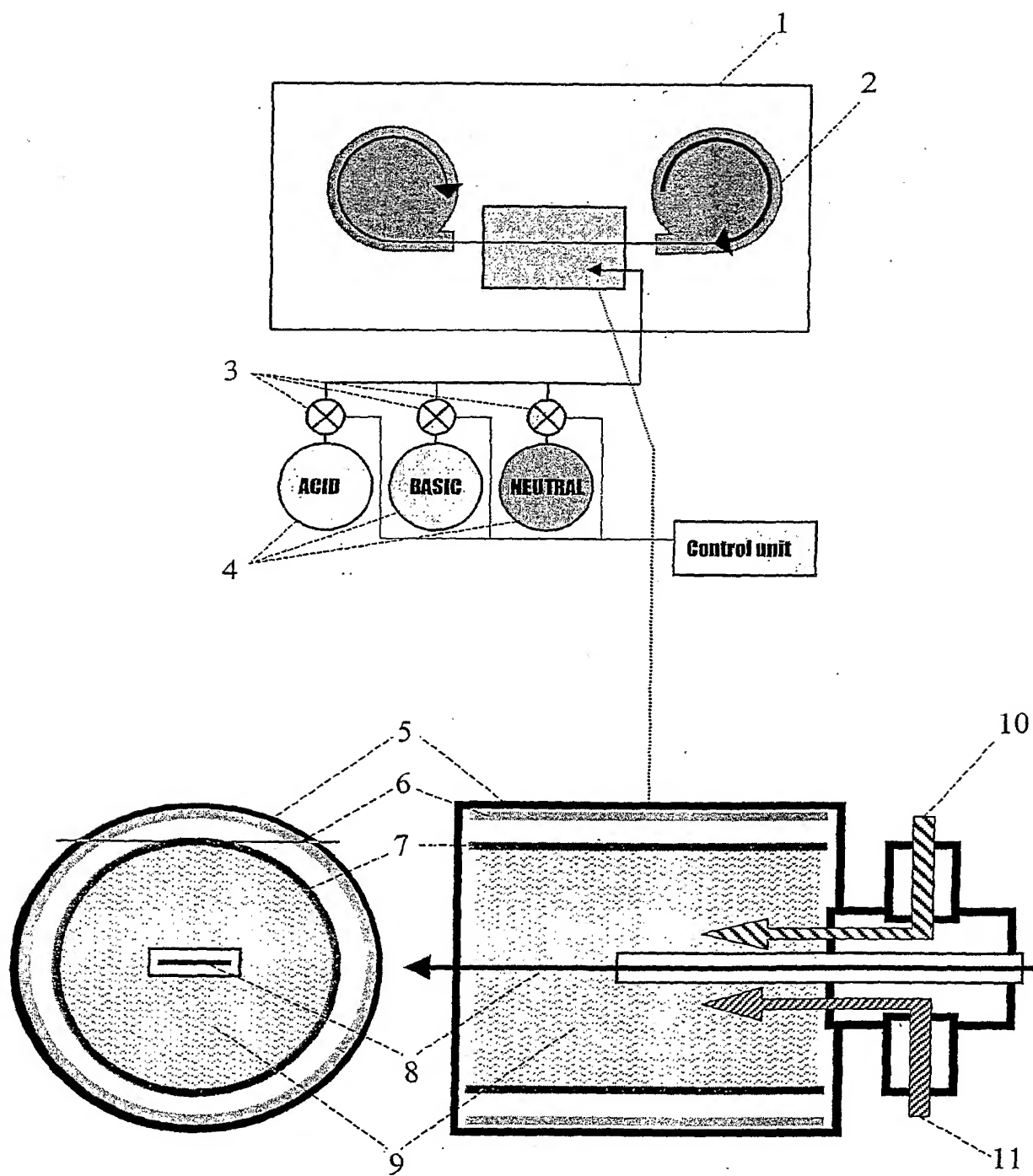


Fig 1.